

Occurrence of food borne pathogen pathogenic E-coli in some types of soft cheese in local market

ABSTRACT

The purposes of this practical research to detect the incidence of pathogenic *E. coli* in kariesh cheese, Ddomiatti cheese and Tallaga cheeses. Confirmatively serological identifications are abasic step of the isolated strains of pathogenic *E. coli* were performed. A bout collecting on the land (150) One hundred and fifty random samples of kariesh cheese, Ddomiatti cheese and Tallaga cheeses (50 each) were collected from different shops and street vendors in El Gharbia territories. Samples were bacteriologically examined for the prescence of *E. coli*. The pollutions incidence of *E. coli* in the analyzed samples were 54%, 42% and 32%, respectively. Serotyping of isolated pathogenic serovars of *E. coli* were : O119, O111, O86 & O128 and O124 in Ddomiatti cheese. Genotypic identification of *E.coli* of virulence genes (Stx1,eaeA, fimH) with occuerrence were recorded at percentage of Zero %, 28.5% and 100 % respectively. In Kkariesh cheese, . Tthe detected *E. coli* incidences of in the analyzed samples surely able to causes a public health diseased outbreaks to the consumers and stipulated the local hygienists to insures maximum health's stipulations.

Therefore this work was planned to covers the followings points:1- Isolations, biochemicals and serologically identifications of pathologically pathotypes of Escherichia coli from kariesh, tallagea and Ddomiatti types of cheeses in eEl _Gharbia governorate. 2-Molecular identifications of isolated bacteria (bacterium coli) using PCR technique.

KEY WORDS: *E. coli*, biochemical, in vitro sensitivity, virulence genes, soft cheese.

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1. INTRODUCTION

بداية فقرة Among the dairy products, cheese is considered one of the most public sources of vital nutrients (e.g. vitamins, minerals and proteins) which represent the main part of healthy food (López-Expósito *et al.*, 2012). Nevertheless, cheese is a popular food for human being, sometimes its deterioration by various types of foodborne pathogens may take place. Manufacturing of cheeses is profitable due to high demands of consumers and low industrial risks , and there are many factories and farmers manufacturing it without supervisions and without health licenses (Kousta *et al.*, 2010; Dias *et al.*, 2012). In Egypt. Moreover, Kkariesh cheese is a prevalent type of cheese which contains a higher protein content with a small amount of fats (Hamad, 2015), . Kariesh and is of a low prices so has poor's demands on a large scales in Egyptian cities and Arabian countries, similar to Domiati (Brown , 2004; Abd-Ehamid , 2012; Hegazy *et al.*, 2012). Domiati cheese is one of soft pickled cheese and produced and consumed on a wide scales due to rich in basic supplemental elements and had a pleasant taste ,/strong sharp flavor as well as smooth body and texture. /Tallaga cheese is widely manufactured widely soft cheeses in El-Gharbia territories due to its values, have with nearly neutral salty taste for hypertensive peoples of all ages with and smoothly textures for old peoples have teeth problems . بداية فقرة *Escherichia coli* is one of a genus of family enterobacteriaceae of all warm blooded animals , so confirmative detections constitutes the absences of health supervisions and stipulated canceling of this detected suppliers and doubts of all territories of the cheeses presenters due to the lack of medical bacteriological slandered executions standards (Kousta *et al.*, 2010; Dias *et al.*, 2012). Enteropathogenic *E. coli* (EPEC) are considered the most common bacteria isolated from cheese. EPEC also that cause high morbidity and mortality rates among young and old people (Kousta *et al.*, 2010).

بداية فقرة *E. coli* is environmental resistant fecal and urinal indicators, which has a bad health impacts on both human and animals species. Thus, this type of bacteria can deteriorate the milk particularly raw milk and other milk products as a result of poor hygienic measures (Lara *et al.*, 2016; Garbaj *et al.*, 2016).

Enteropathogenic *E. coli* (EPEC) is considered the most common bacteria isolated from cheese. *E. coli* constitutes public hazards of living creatures especially humans (likes meningoencephalitis, pyelonephritis, hepatitis, pneumonitis, pleurisy, pericarditis, peritonitis, osteomyelitis, appendicitis). *Shigatoxin producing E.coli* (STEC) have a potent two verocytotoxic toxins which destructed all functional organic epitheliums especially enteritis in all vital systems leading to dehydrations or organics failures and causing peracute inflammations, bloody diarrhea, haemolytic uraemic syndrome, of kidneys leading to sudden failures with red urine and encephalitis's and deaths (Pennington, 2010). STEC strains is a world public hazard pathogen have two genes Stx1 and Stx2 may with each other or separately but constitutes powerful diseased virulence's genes and surely causing shigellosis similar diseases especially in weakly or hypo immunized individuals suffering from chronic diseases or viral diseases aging consumers. (Lindgren *et al.*, 1993), (Muniesa *et al.*, 2004 and Gyles, 2007).

بداية فقرة STEC detected serotypes and genotypes is surely classified food-borne pathogens because of destructions of all parenchymatous organs causing breakdowns, and suffering and outbreaks of deaths. (Bach *et al.*, 2002, Blanco *et al.*, 2003). The most common serovars world wide were O26, O91, O103, O111, O128, O113 and O145 are responsible for up to 20 to 50% of all STEC infections (Johnson *et al.*, 2006). The somatic serovars causing seriously humans chronic diseases in the developing countries were 26, 91, 103, 111, 113, 121, 145 and 157 (Karmali *et al.*, 2010; Pizarro *et al.*, 2014). *Eae A* gene it is an intimin secretion gene that enhance

attachment and colonizations of *Escherichia coli* in the intestinal epithelium leading to severe cellular destruction and severe infections and severe symptoms like profuse diarrhea and ulcerative colitis (*Ibrahim et al .*, 2019).Urinary specific *Escherichia coli* is genetically based on the fimbrial structural type 1 gene which responsible for most UTIs diseased cases. Uropathogenic *E. coli* (UPEC) strains is the hidden causative bacteria of vaginitis and causes vaginal hemorrhage especially in females based on honest experimental results whom real medical scientists. The most virulence factor dependent upon the UPEC include adhesions type 1 fimbriae encoding by fim H gene (Zohreh et al 2014).

2-Material and Method

The study was undertaken to isolate pathogenic *E.coli* from various types of cheese

2.1. Sampling:

One hundred and fifty random samples of soft cheese (50 samples of Kkariesh cheese – 50 samples of Tallaga cheese – 50 samples of Domiatti cheese) (50 samples of each of kariesh, domiatti and tallaga cheeses) were collected randomly from small dairies, local super markets, farmers house, shops and street vendors in different localities at El-Gharbia Governorate, Egypt. The selected samples were delivered directly to the institute in an ice – box under aseptic conditions without delay and then examined bacteriologically.

The bacteriological examinations of samples were carried out in Animal Health Research Institute Tanta, while serological examinations was done in Animal Health Research Institute Dokki.

2-2. 2.2. Bacteriological examination:

2.2.1. Isolation of *E. coli* Preparation of samples: (مرجع اكتب)

5 Five grams of samples of each cheese sample (Kariesh samples - Tallaga cheese- Domiatti cheese) were added to 25ml sod. Citrate and blended for two minutes. examination of *E.coli* by this tests.

2-2-1.1. MacConkey's broth test (acid and gas test):

2.2.2. Isolation of *E.coli*:

Two ml of each blended prepared samples are added to MacConkey broth with ibin Dorf tubes and incubated for 24hours at 37°C for the detection of acid and gas if it is present as indicative indicated for the presence of *E. coli* (المرجع).

2-2-1.2. Eosin methylene blue media test:

بداية فقرة From the positive MacConkey's broth test with yellow colorations and (acid) and gas results are cultured on the Eosin Methylene Blue (EMB) agar plates, which it is as a selective media for *E.coli*, then incubated at 38 °C for 24 hours. Typical colonies of *E. coli* appeared greenish metallic with dark purple center (المرجع). Suspected colonies were detached by bacteriological loop and transferred to nutrient agar slopes and then incubated at 38 °C for 24 hours. The purified colonies were subjected for further morphological, biochemical and serological examination.

2.3. Identification of isolates:

2-2-2- 2.3.1. Morphological identification : *E.coli* appear as Gram negative bacilli arranged singly or in short chains (المرجع).

2-2-3- 2.3.2. Biochemical identification: The pure colonies of isolates were identified biochemically according to (Quinn *et al.*, 2002). تكملة فقرة Indole, Methyl red, Vogues-Proskauer, Simmon citrate and Triple sugar iron tests were done. Results are recorded according to the following table:

Table 1

Bacteria	Biochemical tests				
	Indole	Methyl red	Voges-Proskauer	Citrate utilization	TSI
<i>E. coli</i>	+	+	-	-	A/A -ve H ₂ S

2.3.3. Serological Identification of *E. coli* serovars according to (Kok *et al.*, 1996).

- Slide agglutination test were done with suspected colonies with poly valent antisera if positive for agglutination this colonies is confirmatively positive for *Escherichia coli* and the same test were done for positive one with monovalent antisera to detected the somatic antigen because each strain contain only one somatic antigen in their body.

2.4. In-Vitro antibiotic sensitivity of *bacteria isolates* according to Srivani (2001).

As shown in Table(1, 6). Subcultures from the isolates were prepared and the test was applied as follows:

A smooth single colony was inoculated in 5 ml nutrient broth and incubated at 37°C for 18 hrs, then turbidity was adjusted to 0.5McFarland contain (1.5×10^8) colony forming unit/ml, then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically and the plates were allowed to stand for 15 minutes at 37°C for dryness. Then the inoculated plates were over laid with antibiotic discs using a sterile forceps, the discs were distributed in a manner where the distance among them was optimum and away from the edge of plate to avoid overlapping of inhibition

zones and give more wide area for the zone of inhibition. The inoculated plates were incubated at 37°C for 24 hours. The Inhibition zones, in mm were measured and scored as sensitive, intermediate and resistant categories with the critical break points recommends by CLSI (CLSI, 2011)., as shown in the next table

Table 2: Antimicrobial discs, concentration or antibiogram profile of antibiotic susceptibility

Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Sensitivity disc content (µg)	Antimicrobial agent
18 or more	13-17	12 or less	30	Chloramphenicol (C)
20 or more	15-19	15 or less	5	Ciprofloxacin (CP)
23 or more	14-22	13 or less	15	Erythromycin (E)
4 or more	1-2	05 or less	5	Enrofloxacin(ENR)
17 or more	13-16	12 or less	30	Neomycin (N)
128 more	64	32 or less	300	Nitrofurantoin (F)
19 or more	15-18	14 or less	30	Oxytetracycline(OT)

2.5. Detection of virulence factors of *E. coli*..

Extraction of DNA according to QIA amp DNA mini kit instructions, Preparation of PCR Master Mix According to Emerald Amp GT PCR master mix (Takara) Code No. RR310Akit, Cycling conditions of the primers during cPCR (temperature and time conditions of the two primers during PCR according to specific authors and Emerald Amp GT PCR master mix (Takara kit) they have specific sequence and amplify a specific product as shown in Table (3), DNA Molecular weight marker the ladder 100bp was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded and agarose gel electrophoreses (Sambrook *et al.*, 1989).

Table 3: Oligonucleotide primers sequences Source: Metabion (Germany).

Gene <i>E.coli</i>	Sequence	Amplified product	Reference
<i>Stx1</i>	ACACTGGATGATCTCAGTGG	614 bp	Dipineto <i>et al.</i> , 2006
	CTGAATCCCCCTCCATTATG		
<i>fimH</i>	TGCAGAACGGATAAGCCGTGG	508 bp	Ghanbarpour and Salehi, 2010
	GCAGTCACCTGCCCTCCGGTA		
<i>eaeA</i>	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisi-Johnson <i>et al.</i> , 2011
	GCCTTCATCATTTTCGCTTTC		

3.RESULTS:

The morphological characters of the colonies on Eosin Methylene Blue were brilliant green. A total of 64 bacterial isolates were obtained from the water samples. The results of the morphological and biochemical tests are summarized in Table 1.

obtained from examined samples, of them 27 bacterial isolates from kariesh cheese, and 21 bacterial isolates from Ddomiatti cheese . and 16 bacterial isolates from Tallaga cheese. *E.coli* isolated from kariesh , domiatti and tallaga cheeses with an incidence of 54%, 42 and 32 respectively as shown in table 4.

E. coli isolated from domiatti cheese with an incidence (42%) . *E. coli* isolated from Tallaga cheese with an incidence (32%) as shown in (Table 3).

E. coli give positive results to Indole, Methyl red and negative results to Vogues-Proskauer and simmons citrate. *E. coli* give yellow slant and yellow bottom without H₂S production as shown in Table (4).

five *E. coli* isolates were serotyped, four from kariesh cheese and one from tallaga cheese, serological identification revealed that five isolates from kariesh cheese were belonging to (O119 :H7, O111 :H8, O86: O18: H1 :K7 and O128). One isolates from domiatti cheese belonging to (O124) (table 5) وضح المكتوب الجدول به 6 عترات... تاكد من عدد العترات ومصدرها

ولماذا هذا العدد فقط من العترات التى تم اختبارها ؟

Table (1): Antimicrobial discs, concentration or antibiogram profile of antibiotic susceptibility of their action on the isolated *E.coli*.

Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Sensitivity disc content (µg)	Antimicrobial agent
18 or more	13-17	12 or less	30	Chloramphenicol (C)
20 or more	15-19	15 or less	5	Ciprofloxacin (CP)
23 or more	14-22	13 or less	15	Erythromycin (E)

		less			
4 or more	1-2	05	or	5	Enrofloxacin(ENR)
		less			)
17 or more	13-16	12	or	30	Neomycin (N)
		less			
128 more	64	32	or	300	Nitrofurantoin (F)
		less			
19 or more	15-18	14	or	30	Oxytetracycline(O
		less			T)

Table (2): Oligonucleotide primers sequences Source: Metabion (Germany).

Gene <i>E.coli</i>	Sequence	Amplified product	Reference
<i>Stx1</i>	ACACTGGATGATCTCAGTGG	614 bp	Dipineto <i>et al.</i> , 2006
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<i>eaeA</i>	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisi-Johnson <i>et al.</i> , 2011
	GCCTTCATCATTTTCGCTTTC		

Table (4): Incidence of *E. coli* isolated from soft cheeses samples in El-Gharbia - Governorate Egypt.

Examined samples	E.coli positive samples-isolates	
	%	<i>E.coli</i> number
Total cheese n=150	42.5	64
Kaiesh cheese n=50	54	27
Domiatti cheese n=50	42	21
Tallaga cheese n=50	32	16

Table (4) Biochemical tests of *E. coli*:

Bacteria	Biochemical tests				
	Indole	Methyl red	Voges-Proskauer	Citrate utilization	TSI
<i>E. coli</i>	+	+	-	-	A/A -ve H ₂ S

Table (5): Results of serological identification of *E.coli* isolates.

Serial	Identified Bacterium	Serodiagnosis	Strain
1	<i>E.coli</i> (K3)	O1119 : H7	EPEC
2	<i>E.coli</i> (K1)	O111 : H8	EHEC
3	<i>E.coli</i> (K2)	O 86	EPEC
4	<i>E.coli</i> (K5)	O18 : H7	ExPEC

5	<i>E.coli</i> (K6)	O128	EIEC
6	<i>E.coli</i> (D3)	O124	EPEC

K =Kariesh cheese , D=Domiatti cheese , T= Tallaga cheese .

Table (6):Antimicrobials sensitivity results for *E. coli* isolates according to Srivani (2001).

Antimicrobial agent	Diffusion zone break point (mm)	<i>E.coli</i> D3 O128	<i>E.coli</i> K3 O119	O186 <i>E.coli</i> K2	<i>E.coli</i> K1 O111	<i>E.coli</i> K5 O118	Percent Sensitivity
Ciprofloxacin (CIP)	(mm) 12	S(20)	S(28)	S(29)	S(27)	S(32)	100%
Enrofloxacin (ENR)	(mm)0.5	S(8)	S(9)	R(0.3)	S(7)	R(8)	83.33%
Chloramphenicol (C)	(mm)15	S(23)	S(26)	R(7)	R(8)	I(17)	59%
Oxytetracycline (OT)	(mm)14	S(22)	S(21)	R(11)	S(25)	S(24)	83.33%
Neomycin (N)	(mm)12	I(14)	R(9)	R(8)	R(9)	R(7)	%8
Neutrofurantoin (F)	(mm)32	R(25)	S(145)	R(24)	R(19)	S(149)	50%
Erythromycin (E)	(mm)13	R(9)	I(17)	R(9)	S(28)	R(7)	25%

S= sensitive

R= Resistant

I =
intermediate

K= Kariesh cheese

D=Domiatti cheese

O128 اين اختبار الحساسية لها؟

Table (7): Results of molecular identification of *Stx1*, *eae A* and *fim H* genes of *E. coli* isolates.

Results			Sample isolate	Target MO
STX	eaeA	fimH		
1				
-ve	-ve	+ve	1	<i>E. coli</i>
-ve	-ve	+ve	2	
-ve	+ve	+ve	3	
-ve	+ve	+ve	4	
-ve	-ve	+ve	5	
-ve	-ve	+ve	6	
-ve	-ve	+ve	7	

Pictures of PCR :

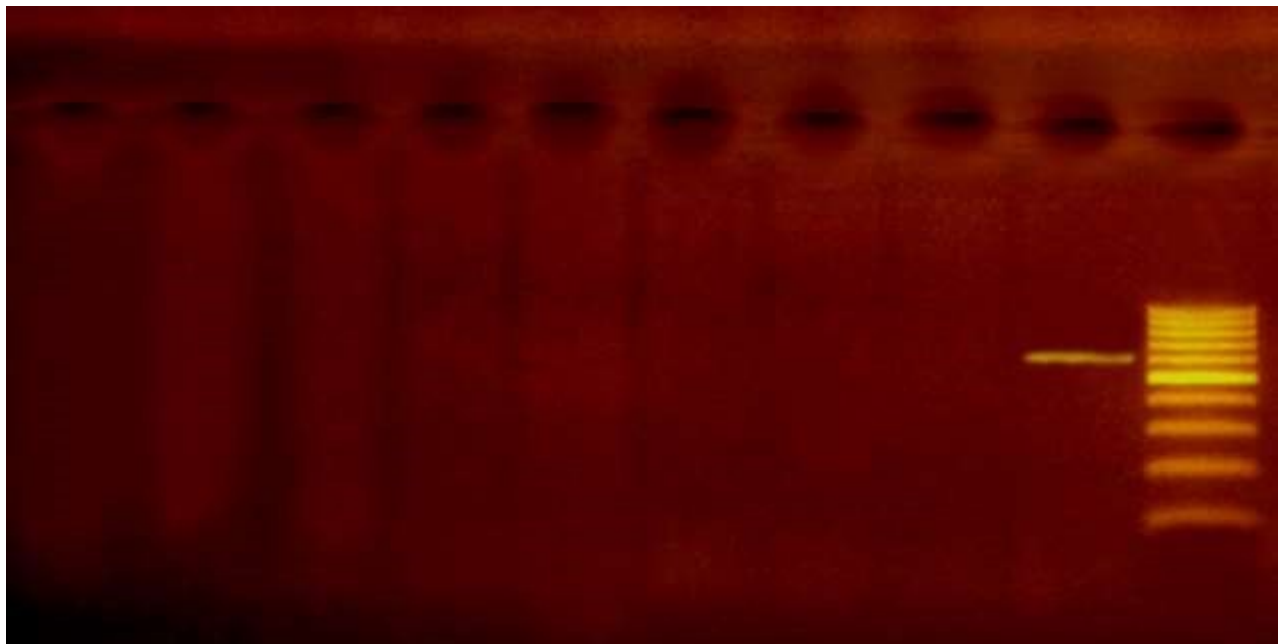


Figure (1): Agarose gel electrophoresis of PCR amplified products of virulence gene. For *stx1* gene was negative .

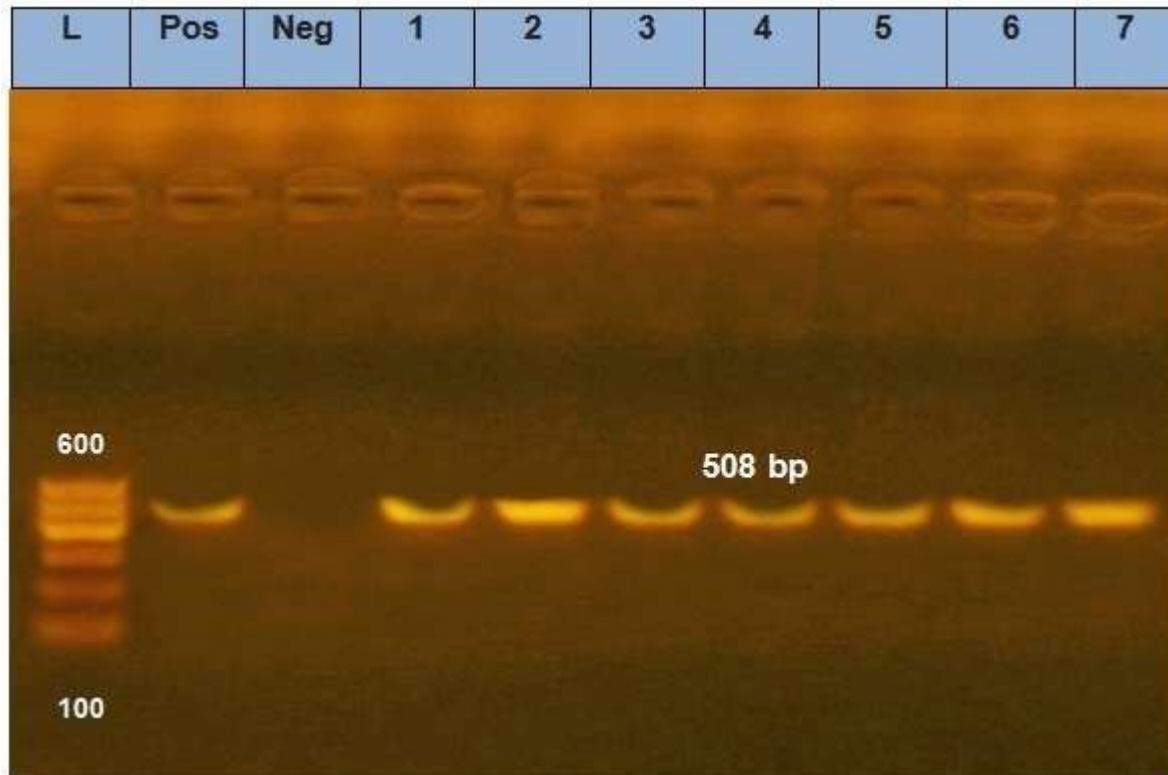


Figure (2): Agarose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 1,2,3,4,5,6,7: *fimH* virulence gene of *E. coli*. The size in base pairs (508bP) of PCR product is indicated for the bands.

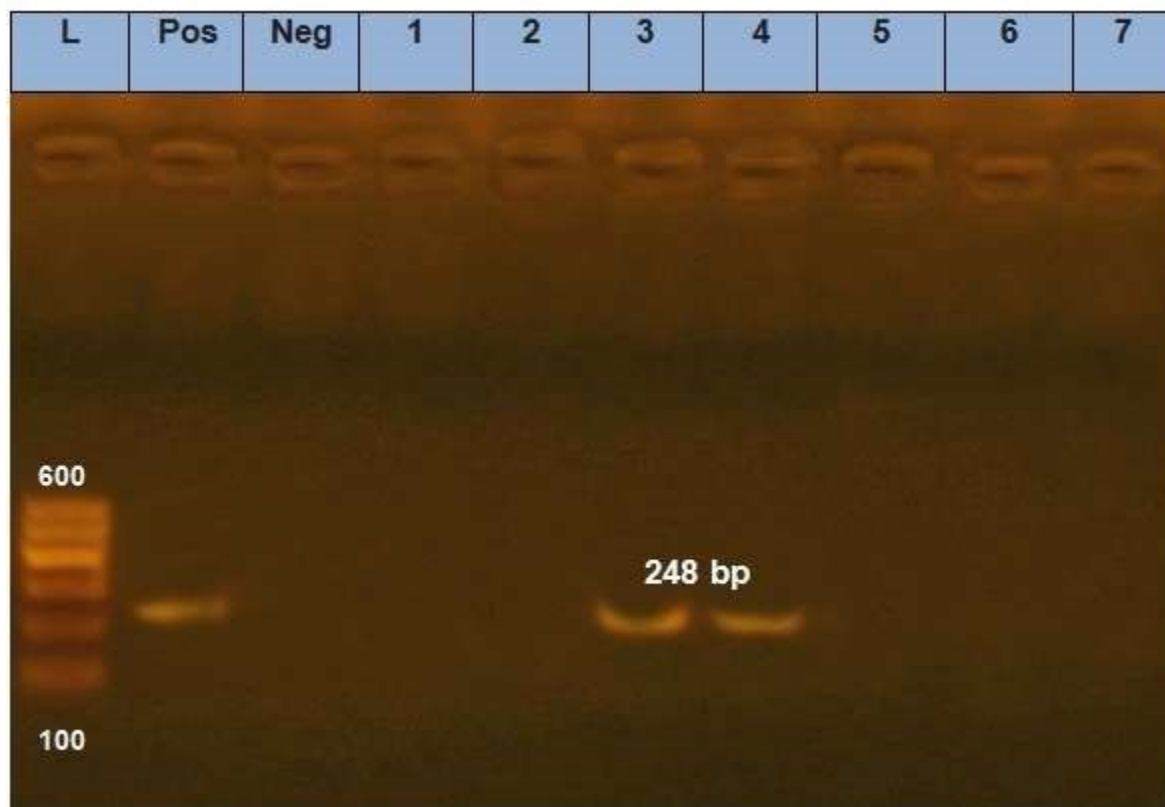


Figure (3): Agarose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 2,3: *eaeA* virulence gene of *E. coli*. The size in base pairs (248bP) of PCR product is indicated for the bands.

4. DISCUSSION

بداية فقرة Illness caused by enteropathogenic *E. coli* can range from self-limited watery diarrhea to life threatening manifestations such as hemorrhagic colitis, hemolytic uremic syndrome, thrombocytopenic purpura and may lead to death (alexander and prado, 2003).

Recently there is additional concern about the presence of *E.coli* in cheeses because of the ability of some strains to cause food borne illness and lead to high incidence of food outbreaks .

1- Kariesh cheese : table (3)

4.1. Incidence of *E.coli* in the examined soft cheeses (table 4):

Inspection of of kariesh cheeses table 4 revealed that *E. coli* could be isolated from examined Kkareish cheese samples with incidence rate of 54%. Nearly similar finding was reported by El-bajory *et al.*, (2015), El-nahas., *et al* (2015), Nagah *et al.*, (2012). Higher incidence was reported by Ibrahim *et al.*, (2019), Ombark *et al.*, (2016),El-kosi (2001),Mohammed (2015) while lower incidence was reported by El-Sayed *et al.*, (2011), Zeinhom (2011), Hosney *et al.*, (2011), hefney(2012), Abd EL- Latif, eman (2012), Ahmed (2012), Brooks *et al.*, (2012), Nagah *et al.*, (2012), Samaha *et al.*, (2012), Elhadidy and Mohammed (2013), Bonyadian *et al.*, (2014), Abo Zeed (2014), Metwally and Fatma (2015), El nahas *et al.*, (2015), Gamal *et al.*, (2 015), Jehan *et al.*, (2015), Gamal and Soad (2016), Sameh awad (2016) and Lotfy *et al.*, (2017).

Damietta cheese

It is evident that *E. coli* could be isolated from examined Ddamietta cheese samples with incidence rate of 42%. Nearly similar finding was reported by Ozen *et al.* (2011), Ibrahim *et al* (2019) while lower incidence was reported by Ozen *et al.*, (2011), Ibrahim *et al.*, (2019) while lower incidence was reported by Zeinhom (2011), Hefney (2012), Nagah *et al.*, (2012), Elhadidy and Mohammed (2013), Gamal and Soad (2016), Jehan *et al.*, (2015) ,Gamal *et al.*, (2015), Elbassary (2006) , El-bajory *et al.*, (2015) and Lotfy *et al.*, (2017).

Tallaga cheese :

It is evident from the table 4 before that *E. coli* could be isolated from examined tallaga cheese samples with incidence rate of 42 32%. Nearly similar finding was reported by El-Sayed *et al* (2011), while lower incidence was reported by, Ahmed (2012).

بداية فقرهContamination of milk and milk products, with *E. coli* is largely due to unhygienic condition during production, processing, handling, and distribution. *E.coli* is a good indicator of fecal pollution as it exists in the

normal microflora of the intestinal tract of humans and warm blooded animals(المرجع).

Serological typing of isolated *E. coli* strains: table : (5)

4.2 Serological typing of *E. coli* isolated from soft cheese samples: (table 5)

It is evident from the results recorded in serotyping that some of isolated *E. coli* strains (64 strains isolates) could be serologically identified the pathotypes of *E.coli* isolates from some types of soft cheeses that could be identified serologically were : (O111) (EPEC) Enteropathogenic Escherichia coli, (O86) (EHEC) Enterohemorrhagic Escherichia coli ,(O 18) (Ex PEC) Extera intestinal pathogenic Escherichia coli ,(O128) Atypical EPEC Escherichia coli , (O124) (EIEC) Entero Invasive Escherichia coli . مختلف عن الجدول .

2- Sensitivity Test of Isolated *E.coli* in Examined Soft Cheese : (Table 6) according to CLSI :(2018):

4.3. Sensitivity of *E.coli* isolated from the examined soft cheese samples: (table 6)

Isolates show sensitivity to the aAntibiotics used in this test as 100% for Ciprofloxacin CIP(10 Mg) (100%) , 83.3% for Enrofloxacin E ENR (5 Mg) (83.3%), 83.3% for Oxytetracycline OT (30 Mg)(83.3%), 50 % for Chloramphenicol C (30 Mg) (50%), 50 % for Nitrofurantoin F (300 Mg) (50%) , 8% for Neomycin N (30 Mg) (8 %), , and 25% for Erythromycin (15 Mg) (25%) . It is an advice tThe common mistakes of antibiotics in the veterinary field in treating of colibacillosis in animals or colisepticemia in humans it is the main cause selecting the resistance of pathogenic types of Escherichia coli becouses as the selected antibiotics not the most killer one .

3- Polymerase Chain Reaction (PCR): (Table 2,7): Genotypic identification of *E.coli* of virulence genes (Stx1,eaeA, fimH) with occurrence percentage Zero % ,28.5%and 100 % repectively.

4.4. Genotypic identification of *E.coli* virulence genes (Stx1,eaeA, fimH) (table7, figures 1,2 and 3)

Stx1 gene It is a/shgia toxin potent verocytotoxic and cause sever illness (المرجع) Its prescence in our isolates by PCR was zero % and not found in all *E.coli* isolates. Nearly similar findings was reported by *Taha et al(2019)* while higher incidences findings were reported by *Ibrahim et al (2019)*, *Sabrey et al(2008)*, *Hussien et al (2019)*, *El-hadidy and Mohamed (2012)*, *Ombark et al (2016)*, *Chaleshtori et al 2017*. الغى الخط المائل

2-Eae A gene it is an intimin secretion gene that enhance attachment and colonizations (المرجع):it was detected Its prescence in our *E coli* isolates by PCR was at percentage of 28.5 % of *E.coli isolates* : while lower incidences findings were reported by *Ibrahim et al., (2019)*, *Sabrey et al.,(2008)*, *Hussien et al., (2019)*, *El-hadidy and Mohamed (2012)*, *Chalechtori et al., (2017)*.

3-Fim H gene it is responsible for biofilm formation and enhance infection of Uropathogenic Escherichia coli and the structural rule of type 1 fimbria and responsible of about 92 % of the pathogenicity and diseased cases in human patients suffering from Urinary tract infections (المرجع) Its presence in our isolates by PCR was 100 % of *E.coli* isolates. Similar results and findings were reported by *Garofalo et al (2007)* while lower incidences findings were reported by , *Watts et al (2010)*, *Mladin et al (2009)*, *Arabi et al (2012)*, *Tarchouna et al (2013)*, *Sabrey et al(2008)*, , *Zohreh et al (2014)*, *Hussien et al (2019)*.

According to Egyptian standard (2005) which stipulated that milk and milk products must be free from *E. coli*, (المرجع). بضاف فى المراجع

5. CONCLUSION

Practical honest bacteriological examinations executed in animal health institute revealed that the soft cheeses ready for sales and consumptions exhibited in the market of Eel Gharbia governorate revealed that show high Escherichia coli presence percentage incidence in the cheeses exhibited in the market ready for sales and consumptions due to weak or absence of responsible authorities . Milk used for cheese manufacture must of good qualities and from dairy cattle's free from mastitis, . farmers manufactured cheeses must free from diseases especially Escherichia coli infections all equipments must be cleaned and sanitized by veridical, bactericidal and fungicidal detergents . Finally, the present study allow to conclude that : Kkariesh cheeses more contaminated with pathogenic Escherichia coli with heath fatal or chronic complications likes hemorrhagic colitis , hemolytic uremic syndrome and meningoencephalitis and even death. The serotyping confirmed the present of more virulence serotyping world wide as O 111, The best antibiotics in treatment to saves life's were detected as follow ciprofloxacin , enrofloxacin and oxytetracycline , so strict hygienic measures during the production , transportation and selling of un pasteurized kariesh cheeses and regulatory checking of the workers is of great importance to prevent the contaminations with Escherichia coli.

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